EXHIBIT I

LONG TERM FOLLOW-UP OF HYDROXYAPATITE CEMENT (HAC) IMPLANTS FOR CRANIOFACIAL RECONSTRUCTION

108

Roger E. Horioglu*, Craig D. Friedman*, Peter D. Costantino*^
Shozo Takagi^, and Lawrence C. Chow^

*Yale University School of Medicine
New Haven, Connecticut

INTRODUCTION

Hydroxyapatite cement (HAC) is a unique calcium phosphate based material which forms a paste when mixed with water, and sets into a solid implant within 15 minutes. It differs from ceramic hydroxyapatite in that it is produced by direct crystallization of HA in vivo, as opposed to the process of sintering used to produce ceramic HA.[1] HAC has many potential characteristics of an "ideal" implant for craniofacial reconstruction. It is available in large quantities, it can be easily shaped intraoperatively into the defect site, it provides sufficient strength for non-stress-bearing applications, there is no foreign body reaction, and the implant becomes replaced and ingrown by new bone over time.

Previous studies with feline models have shown HAC to be a good candidate for cranioplasty and frontal sinus reconstruction.[2,3] The aim of this study was to further evaluate HAC as a craniofacial reconstruction material in a long term follow-up of 30 months, using physical strength testing, CT imaging, and bone histometry techniques.

METHODS

HAC cranioplasty: After a midline scalp incision, 2.5 cm diameter full thickness parietal craniotomy site was created in three cats using a high speed cutting burr. The defect was completely filled with 100% HAC, HAC allowed to solidify, and then incisions closed. The animals survived for 30 months after implantation. After sacrifice, the calvarium, including craniotomy sites was removed. The specimens were grossly examined and diameters of the remnant implants were measured. Computed tomography of the implant site was performed. Seven cylindrical disks were trephined from the HAC/bone interface area and another seven from nonimplanted areas of skull to be used for diametrile tensile strength(DTS) measurements. The remaining specimen was sectioned into coronal sections for histologic analysis.

Frontal sinus reconstruction: Following a midline scalp incision in one cat, region of the frontal sinus was exposed, and the roof of the left frontal sinus was removed with a micro-oscillating saw. Mucoperiosteum of the sinus was completely removed and the entire sinus was then filled with HAC. After sacrifice and harvesting of the calvarium at 30 months, CT scan of the implanted area was obtained to visualize the cross section. The specimen was then sectioned coronally for histologic studies.

Histology/histometry: Sections from cranioplasty and frontal sinus specimens were stained with Paragon and Von Kossa stains, both of which enable differential visualization of bone, osteoid, and HAC. Histometric measurements were performed(using OsteoMeasure Version 2.1 to quantitate the volume fraction of bone components at three different locations: Central portion of the implant (C), implant/bone interface (I), and normal calvarial bone (B). In addition, fractional volume of implant in bone was calculated by measuring total area of implant in a series of cross sectional slides. From this, % total HA resorption/replacement was determined.

RESULTS

All four cats survived the course of the experiment. There were no postoperative complications, infections, or implant extrusions.

Gross examination: On the cranioplasty specimens, HAC implants were well integrated into the surrounding bone. On the external surface, HAC implant was visible as a circular area that appeared lighter than the surrounding bone. The size of the implanted area appeared markedly reduced. The internal surface of the implanted area appeared completely covered by new ingrown bone and implant couldn't be visualized. The skull surface contour was well maintained both externally and internally, as well as the shape and volume of implant and surrounding bone.

Frontal sinus specimens similarly had good integration of HAC implant with well-maintained contour, shape, and volume.

Diameters of the three implanted areas on the cranioplasty specimens were measured on three different axes, and an average diameter of 11.1 mm (SD 2.4 mm) was calculated, compared to the initial craniotomy defect of 25 mm.

Cross-sectional imaging: CT scan with coronal sections through the implanted areas of cranioplasty and frontal sinus reconstruction specimens showed that majority of HAC implant was resorbed and replaced by new bone. Shape and volume of the implanted areas were well maintained.

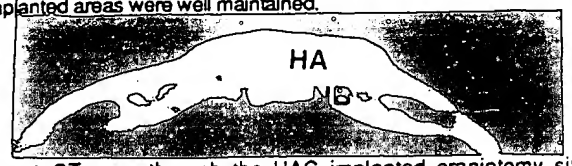

Fig. 1 CT scan through the HAC implanted craniotomy site. HA=remnant HAC, NB=new bone.

Physical strength evaluation: DTS measurements of HAC/bone disks from the interface were within the range of normal calvarial bone as shown below:

Table 1

DTS Normal bone(control)	DTS HAC/bone interface
10.6 +/- 3.9 psi	12.3 +/- 4.6 psi

Histologic/histometric analysis: Paragon and Von Kossa stained slides of specimens revealed that HAC is well integrated with the surrounding bone and that there is active resorption and bone regeneration taking place. On the frontal sinus sections, HAC, which once filled the entire left frontal sinus, has been reduced markedly in size and replaced by new osseous tissue (Fig. 2).

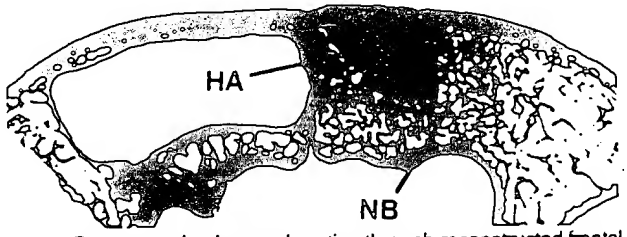

Fig. 2 Paragon stained coronal section through reconstructed frontal sinus. HA=implant, NB=new bone.

Bone histometry results for both cranioplasty and frontal sinus specimens are as shown in Table 2.

	Cranioplasty			Frontal Sinus Rec.		
	B	C	I	B	C	I
Bone	73.4/14.1	35.9/30.1	72.9/11.3	41.2/32.4	3.0 /11.9	61.8/19.4
Osteoid	0.2/ 0.05	0.3 /0.2	0.8 /0.6	0.3 /0.2	1.0 /1.1	1.47 /0.8
Soft tissue	26.4/14.1	15.9/12.5	21.2/14.8	8.5 /32.6	5.7 /8.5	6.8 /19.7
HAC implant	0	47.8/36.7	5.0/ 8.6	0	80.2/17.5	0

Table 2: % Volume Fraction of Bone Components at 30 months (value/SD).B=normal bone, C=implant center, I=interface.

Total implant resorption/osteoconversion was calculated as 81.9% (SD 5.1) for cranioplasty and 82.3% (SD 6.3) for frontal sinus reconstruction specimens.

DISCUSSION

Our long term results at 30 months indicate that HAC is an excellent bone substitute for craniofacial reconstruction. Interfacial DTS measurements indicate that the implant site is sufficiently strong enough for reconstruction of non-stress-bearing craniofacial skeleton and equivalent to control skull bone. HAC becomes progressively resorbed and replaced by real bone over time to a significant extent. Amount of HAC resorption/bone replacement was greater than 80% at 30 months. More importantly, this coupling of resorption and osseous replacement occurs with good preservation of shape and volume.

REFERENCES

1. Costantino P et al. Arch Oto Head Neck Surg. 1991; 117:379-384.
2. Friedman C et al. Arch Oto Head Neck Surg. 1991;117: 385-389.
3. Costantino P et al. Plast Reconstr Surg. 1992;90:174-185.

*Dept.Oto-Head & Neck, Loyola Univ Medical School, Maywood,IL.
^ADA Health Foundation; PRC Lab, NIST, Gaithersburg, MD.